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14. ABSTRACT: This Award funded the initiation of a mentored research experience in ovarian cancer biology at the Dana Farber/Harvard Cancer Center. The primary aims, articulated in the Statement of Work, included creating a mechanism to identify and select outstanding postdoctoral fellows who had a commitment to serious multi-year experience in research that was directly related to a topic in or immediately applicable to ovarian cancer. The second aim was to provide a mentored experience for selected fellows. The third aim specified the delivery of feedback to the trainees by mentors and the program PI. The final aim described a rigorous review process for the program. These aims are all being addressed. Of the four senior post doctoral fellows selected to work with Faculty at Harvard Medical School in the fields of oncogenesis, signal transduction, pathology and mouse models and cell biology, one fellow graduated from the program and is successfully transitioning towards an independent academic research career. The vacancy was competed for and filled successfully. A new faculty member with extensive training in biologic models was added to the program to mentor the fellows, who continue to pursue their research productively at 3 different institutions within the Dana Farber/Harvard Cancer Center.

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Progress Report for the DOD Training Grant- DAMD17-03-1-0161

PI: Michael Seiden M.D. Ph.D.

Introduction

This is the year 2 progress report for the training grant supported through the Ovarian Cancer Research Program mechanism, which in turn is supported by the Department of Defense. A program was funded to support the training of three postdoctoral fellows in research. Below is listed the Statement of Work of the Ovarian Cancer Training Grant at the MGH and the accomplishments of each of the aims.

Statement of work

1) Identify and select outstanding postdoctoral fellows for participation in ovarian cancer training program

2) Identify appropriate faculty and mentors for selected postdoctoral fellows

3) Review progress of postdoctoral candidates by Principal Investigator and Executive Committee

4) Review overall Training Program

SOW Aim #1 Identify and select outstanding postdoctoral fellows for participation in ovarian cancer training program

During the second year of the program we continued funding for Dr. Sanja Sale and Dr. Yong Zhan. Dr. Ronny Drapkin graduated from the program and is successfully transitioning towards an independent academic research career in ovarian cancer, as was the main goal of this training program (see below). The vacancy created by Dr. Drapkins' graduation was competed both through letters distributed nationally as well as letters and e-mails distributed throughout the Dana Farber Harvard Cancer Center to its 800 members. In addition, the position was advertised in Cancer Research and Gynecologic Oncology. We received one inquiry from an external candidate, a physician in a medical oncology training program at the University of West Virginia. That individual did not have a well-formed plan for transitioning his research to the Boston/Harvard area and did not formally apply. We received 8 applications internally. Several of the candidates were felt to be competitive but a decision was made to provide funding to Jiangyong (John) Miao, Dr. Miao who was entering his second postdoctoral fellowship year with Dr. Sandra Orsulic. Dr. Orsulic in turn had recently joined the ovarian cancer research program faculty (see below).

This year, Drs. Sale and Zhan will complete their second and final year of support of their training program and two additional spots will be competed. Once again, these spots have been advertised in Cancer Research, letters have gone out to approximately 80 thought leaders across the country and the position has been posted not only through an e-mail to the 800 members of the Dana Farber Harvard Cancer Center but has now also been posted on the new Dana Farber Harvard Cancer Center web site (see www.dfhcc.org under research opportunities). A decision regarding the new fellows supported by this program is anticipated sometime in late May.

SOW Aim #2 Identify appropriate faculty and mentors for selected postdoctoral fellows

Sandra Orsulic was added on to the new faculty to the training program (Biosketch attached in appendix). Dr. Orsulic has extensive training in several biologic models including drosophila and mouse genetics and is the first investigator to describe a mouse model for ovarian cancer, which was published in Cancer Cell in collaboration with her mentor Dr. Harold Varmus. Dr. Orsulic was recruited to the Division of Molecular Pathology in the Massachusetts General Hospital Cancer Center approximately three years ago and currently is a funded investigator through the DOD, NCI (she has R01 support), the Mouse Models of Human Cancers Consortium, and the Dana Farber Harvard Cancer Center Ovarian Cancer SPORE. Her main emphasis is on the use of mouse models of ovarian cancer to better understand the biology that underpins ovarian carcinogenesis.

SOW Aim #3 Review progress of postdoctoral candidates by Principal Investigator and Executive Committee

The principal investigator has met several times with all the mentees and sees them monthly at the Ovarian Cancer Basic Science Seminar that is under the direction of Dr. Orsulic. All have presented at the seminar series except for Dr. Miao who will present later this spring.

Graduated Fellow (2003-2004):

Dr. **Ronny Drapkin** left the ovarian cancer training program when he received funding from the Ovarian Cancer Research Foundation through a faculty transition grant. Subsequently he has received funding from the National Cancer Institute through the now highly competitive K08 funding mechanism. He currently has an offer to join the faculty as an independent investigator at the Massachusetts General Hospital but also is interviewing at other academic positions across the country.

A description of his research is presented in the paragraph below. Copies of his listed manuscripts are included in the appendix.

Drapkin Research Abstract

Given that early stage ovarian cancer is largely curable, new approaches to early detection are needed to achieve lower ovarian cancer morbidity and mortality. A sensitive and specific screening test that could detect ovarian cancer at a curative stage has yet to be developed. The application of cDNA and oligonucleotide microarrays analyses to ovarian cancer has resulted in the identification of many genes that are overexpressed in primary tumors and ovarian cancer cell lines. With the aim of identifying a set of genes that are consistently overexpressed in ovarian cancer, we integrated the results of 10 transcript-profiling studies into a database that contains only the genes that overlap between studies. The database includes Mucin 1, Mesothelin, CD9, CD24, EpCAM, and HE4, among others (Drapkin et al., 2004). The expression of each corresponding gene product was confirmed by immunohistochemistry on a collection of ovarian cancer tissue samples.

Among the genes most commonly identified in gene expression profiles of epithelial ovarian carcinomas (EOCs) is the gene for human epididymis protein 4 (HE4). To ascertain its clinical utility, we performed a comprehensive assessment of HE4 protein expression in benign and malignant ovarian

and non-ovarian tissues by immunohistochemistry. In comparison to normal surface epithelium, which does not express HE4, we found that cortical inclusions cysts (CICs) lined by metaplastic Mullerian epithelium abundantly express the protein. This was true of other EOC tumor markers that we studied (Drapkin et al., 2004). The expression of HE4 in tumors was restricted to certain histological subtype: 93% of serous and 100% of endometrioid EOCs expressed HE4, while only 50% and 0% of clear cell carcinomas and mucinous tumors, respectively, were positive. Tissue microarrays revealed that the majority of non-ovarian carcinomas do not express HE4; consistent with our observation that HE4 protein expression is highly restricted in normal tissue to the reproductive tracts and respiratory epithelium. HE4 is predicted to encode a secreted protein. Using RT-PCR we identified ovarian cancer cell lines that endogenously over-express HE4. Cultured media from these cells revealed a secreted form of HE4 that is N-glycosylated. This observation is consistent with the recent report that HE4 circulates in the bloodstream of patients with EOC. Therefore, HE4 is a secreted glycoprotein that is overexpressed by serous and endometrioid EOCs. Its expression in CICs suggests that formation of Mullerian epithelium is a prerequisite step in the development of some types of EOCs (Drapkin et al., 2005). Studies are in progress to determine the utility of HE4 as a serum biomarker for early detection. Our latest studies also indicate that HE4 is required for ovarian cancer cell growth and thus, is more than just an innocent bystander. Future studies will address the specific contributions of HE4 to ovarian cancer cell growth.

Drapkin Publications during DOD funded support:

1) Drapkin R, Crum CP, Hecht J. Expression of candidate tumor markers in ovarian carcinoma and benign ovary: evidence for a link between epithelial phenotype and neoplasia. *Human Pathology* 2004, 35: 1014-1021.

2) Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, Hecht J. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res* 2005; 65: 2162-2169.

Current Second Year Fellows (2003-)

Second year fellow, **Dr. Sanja Sale** remains in the laboratory of Dr. John Blenis and continues to focus on the role of the M-Tor pathway in ovarian carcinogenesis.

Sale Research Abstract

mTOR *(mammalian target of rapamycin)* is an evolutionary conserved serine/threonine kinase that integrates inputs from nutrients, growth factors and cellular energy status and coordinates cell growth and proliferation via the regulation of protein synthesis. Increased rate of translation is essential for maintenance of rapid rates of cell division and is thus an interesting target for therapeutic intervention in malignant diseases. The preliminary results from ongoing clinical trials with rapamycin analogues CCI-770 and RAD001 indicate that inhibition of mTOR signaling has promising antitumor activity with relatively minor toxicities.

The objective of our study was to compare relative contribution of mTOR and its effectors S6K1 and eIF4E to tumor progression and to evaluate their potential as therapeutic targets in epithelial ovarian cancer. In addition we examined their role in modulation of chemosensitivity. Initial analysis was done

in SKOV3 cells previously shown to have constitutively active Akt/mTOR pathway due to the low PTEN expression. Retroviruses were used to generate cell clones stably expressing shRNAs targeting the above mentioned kinases or scrambled-control shRNA. At least two distinct shRNAs were tested for each target. Those inducing the most potent knockdown were chosen for further analysis. Total amounts and phosphorylation states of targeted proteins were assessed with immunoblotting. MTS assay was used for the analysis of cell viability and proliferation rate.
Preliminary results indicate differential contribution of each target to proliferation rate and chemosensitivity. While rapamycin had no significant impact on paclitaxel sensitivity in nanomolar range, stable reduction of mTOR expression increased sensitivity to paclitaxel several fold.. Cisplatin sensitivity was not significantly altered in any of the stable cell lines. This data is consistent with the recent report showing that rapamycin enhances sensitivity to cisplatin only in tumor cells with wild-type p53.(SKOV3 cells are p53 -null).

Publications: None

Dr. **Yong Zhan** continues to work on the function of MIS and its receptor predominantly in the development of the müllerian epithelium in a female rat. It is felt that MIS and its receptor and associated down stream signaling partners are logical targets for interventions for the treatment of epithelial ovarian carcinoma.

Zhan Research Abstract

Müllerian duct regression during mammalian embryogenesis is critical for male differentiation. In mammalian males, the fetal testes produce and secrete Müllerian Inhibiting Substance (MIS), which causes the Müllerian (paramesonephric) ducts to regress. In the absence of MIS, Müllerian ducts continue to develop and differentiate as the oviduct, uterus, cervix, and upper part of the vagina. Müllerian duct dissolution is initiated by binding of MIS to its specific type II receptor (MISRII) in the surrounding mesenchymal cells. The type II receptor then signals downstream through specific type I receptors and receptor-specific Smads, which remain to be elucidated in MIS signaling.

Dr. Zhan's research demonstrates that expression of ALK2 (a candidate type I receptor), Smad8 and Smad5 (receptor-specific Smads of ALK2), is sexually dimorphic in the rat Müllerian duct mesenchyme during Müllerian duct regression, ALK2 and Smad8 being higher and Smad5 lower in the Müllerian duct mesenchyme of the male. Likewise, if female urogenital ridges of rat embryos are treated in organ culture with purified human recombinant MIS, expression of ALK2 and Smad8 is induced, while Smad5 is downregulated in the MISRII-expressing mesenchymal cells. Differential regulation of the type I receptor and receptor-specific Smads in MIS signaling potentially represents a feed-forward mechanism in order to amplify and localize signaling, as seen by the predominant expression of phospho-Smads in the Müllerian mesenchyme compared with other regions in urogenital system. Furthermore, in cultured male genital ridges, ALK2-specific siRNA blocks Müllerian duct regression. Müllerian duct regression in the males can also be inhibited in organ culture by siRNA targeting Smad8, while siRNA targeting Smad5 accelerates Müllerian duct regression. Thus, ALK2 appears to be the predominant functional type I receptor for MIS signaling in the mesenchyme surrounding the Müllerian duct of the rat, acting downstream through receptor-specific Smads, which differentially enhance or inhibit Müllerian duct regression.

Dr. Zhan has also defined the timing of expression of the signaling components of Müllerian duct regression that dictate when regression occurs, and adapted RNA interference (RNAi) techniques to test functional activity of the components of the MIS signaling pathway ex vivo in organ culture. The experimental system of organ culture can recapitulate, with good fidelity, the morphological events occurring in vivo during the development of genital ridges and Müllerian duct regression. RNA interference adapted to organ culture proved to be a very useful tool in defining the complex of downstream molecules important in MIS signaling, with potential for broader use in dissecting a variety of other localized embryonic tissue interactions.

Publications: A manuscript reporting these results is currently in preparation.

New Fellow- (2004-)

Dr. John Miao is working on the role of Hepsin- a desmosomal junction associated protein that whose expression is frequently altered in ovarian cancer. The role of this protein is still unclear and the functional studies looking at Hepsin are currently underway.

Miao's research abstract

Hepsin is an integral plasma membrane protease that contains a catalytic C-terminal domain facing the cell surface and an N-terminal domain facing the cytosol. Hepsin is widely expressed in different tissues and cell types, with prominent expression in the liver and kidney. The high expression of hepsin is associated with several types of cancer, including ovarian carcinoma. In order to gain insight into its function, we studied the subcellular localization and expression of hepsin in ovarian cancer tissues and cell lines. Immunofluorescence analysis showed that hepsin co-localizes with desmoplakin at the cell membrane, where it is linked to keratin filaments at desmosomal junctions. Surprisingly, hepsin is also localized in nucleoli, where it co-localizes with the nucleolar protein UBF, which is involved in ribosome synthesis. Western blot analysis was used to investigate the protein level of hepsin in mouse and human tumor cell lines. Consistent with the hypothesis that the mature form of hepsin is derived from a single-chain zymogen that is processed by cleavage or by shedding from the plasma membrane, proteins of different molecular weights were observed on SDS-PAGE. In order to study the role of hepsin in tumor progression, we generated mouse ovarian cancer cell lines that express GFP, hepsin or a catalytically-inactive hepsin mutant protein, and showed that hepsin promotes tumor formation and metastases in immunocompetent mice. The proteolytic function of hepsin was required for promoting tumor progression. Together with previous data showing upregulation of hepsin in ovarian tumors, our data strongly imply a role for hepsin in the progression of ovarian cancer.

Publication-None since July 2004

Evaluation of the mentees:

Mentors have provided annual written critiques of the mentees. All three mentees were rated favorably. Dr. Zhan felt the need to focus further on his skills as well as on his written and spoken English. He has now given two research seminars in the Ovarian Cancer Research Program and showed significant improvement between his first and second presentation. There were no substantial criticisms of Dr. Sale or Drapkin. Dr. Miao is due for his first review in the near future.

SOW Aim #4 Review overall Training Program

The award of the Dana Farber Harvard Cancer Center Ovarian SPORE has augmented the training program experience in August of 2004. Dr. Seiden serves as Co-PI of the grant and the Director of the Career Development Program. If Dr. Drapkin is successfully recruited to MGH it is likely he will receive support through this mechanism. Funding through the SPORE and a philanthropic gift from a patient has also provided funding for a visiting Professorship and a fall ovarian cancer symposium.

The training program has received favorable informal reviews from the fellow and faculty and indeed, concerted effort has been focused on augmenting and extending the training program due to the success in finding high quality mentees and mentors. At the current time it is not clear if the DOD will re-compete this program and support through the NCI T32 mechanism has sought. A T32 application was submitted in October 2004 and unfortunately will not be funded in the first round of its review. We anticipate getting written criticisms in a few weeks and will decide the appropriate action regarding resubmission at that time.

Conclusion/Problems:

There have been no significant problems in executing the training program as articulated in the training grant in the statement of work. It has been somewhat disappointing that of the 20 or so applicants who have applied for the four competed positions all but one had been foreign born. This reflects a pervasive issue in biologic sciences and academic centers in that a large majority of individuals seeking prolonged periods of basic science training were born and received their initial scientific training abroad. While these individuals are extremely committed and focused to advancing science and making the most of their training opportunity they do not qualify for a large portion of available research grants including all NCI training grants (K series) and many foundation grants. Dr. Drapkin is a U.S. citizen and successfully competed for a K08 while Dr. Sale and Zhan clearly will have much greater challenges in identifying independent research funding and are much more dependent on training programs such as our DOD award or ongoing funding through grants obtained by their laboratory's personal investigator. Indeed, funding for Drs. Sale and Zhan have not been completely finalized yet.

In addition, the success of the program strongly encourages the PI to find the necessary funds to extend this unique opportunity. Hopefully further funds through the DOD or NCI (via the T32 mechanism) will become available when this program is due to end in the summer of 2006.